Home Office

NON-TECHNICAL SUMMARY

The long-term effects of developmental hypoxia on cardiac function

Project duration

5 years 0 months

Project purpose

- (a) Basic research
- (b) Translational or applied research with one of the following aims:
  - (i) Avoidance, prevention, diagnosis or treatment of disease, ill-health or abnormality, or their effects, in man, animals or plants.

Key words

Heart disease, Prenatal hypoxia, Antioxidants

Retrospective assessment

> The Secretary of State has determined that a retrospective assessment of this licence is not required.

Objectives and benefits

Description of the project's objectives, for example the scientific unknowns or clinical or scientific needs it's addressing.

What's the aim of this project?

The overall aim of the project is to investigate the long-term effects of insufficient oxygen supply to an unborn baby. Our specific aims are;

1. To understand the long-term effects of insufficient oxygen on the structure and function of the unborn heart

2. To study the suitability of a class of drugs called "antioxidants" to protect the unborn heart from a lack of oxygen and prevent the development of cardiovascular disease later in life.

Potential benefits likely to derive from the project, for example how science might be advanced or how humans, animals or the environment might benefit - these could be short-term benefits within the duration of the project or long-term benefits that accrue after the project has finished.

What are the potential benefits that will derive from this project?

The main benefit of the study is the advancement of current understanding of the underlying mechanisms that lead to heart disease in babies that received an insufficient oxygen supply in the womb. We hope to identify novel targets for drug intervention to protect people from developing cardiovascular diseases later in life. All of the findings will be published in peer-reviewed leading scientific and clinical journals as appropriate to ensure wide dissemination of the research findings. The information is of direct benefit to physiologists and clinical cardiologists and will provide key information enabling better management of cardiovascular disease.

Species and numbers of animals expected to be used

What types and approximate numbers of animals will you use over the course of this project?

Approximately 1430 Wildtype rats, and 30000 zebrafish over 5 years

Predicted harms

Typical procedures done to animals, for example injections or surgical procedures, including duration of the experiment and number of procedures.

In the context of what you propose to do to the animals, what are the expected adverse effects and the likely/expected level of severity? What will happen to the animals at the end?

Up to 70 pregnant rats will be placed in a chamber for up to 16 days where oxygen levels will be set between 9-21%. This level of oxygen will cause tissue hypoxia in the pregnant rats (known as prenatal hypoxia) and unborn offspring (known as fetal hypoxia). Most of the pregnant rats subjected to prenatal hypoxia will experience procedures which fall under the mild severity banding, including; 1) transient discomfort from blood sampling needles, 2) transient throat irritation (from intubation) and 3) a transient reduction in water intake. A lesser proportion of pregnant rats will experience symptoms which fall under the category of moderate severity banding, including; 1) a transient reduced activity, 2) a transient reduction in food intake and body weight, and 3) a transient feeling of being unwell from maternal pre-

eclampsia-like symptoms. In addition to maternal adverse effects, most of the pups (up to 550 rats) will experience procedures which fall under the category of moderate severity banding, including; 1) a transient or permanent intrauterine growth restriction (IUGR) which causes a reduction in body weight, and 2) a permanent increase in disease susceptibility. In very rare cases, pups will experience permanent physiological and morphological birth defects. All of the zebrafish in this study (30,000 fish) will experience a transient or permanent reduction in body weight caused by hypoxia. At the end of experimentation, all of the pregnant rats, some of the rat offspring (950 rats) and all of the zebrafish will be humanely killed. The rest of the rat offspring (150 rats) will be delivered to a collaborating establishment for a separate, parallel set of experiments; at the end of these experiments, rats will be humanely killed.

Replacement

State why you need to use animals and why you cannot use non-animal alternatives.

We need to use animals because our study is assessing the long-term effects of prenatal hypoxia (up to a year) which cannot be studied in cell lines, nor can they be suitably modelled using computer simulations. It is not possible to use human volunteers because human tissue is of limited availability. Lastly, we cannot use non-protected animal alternatives because we wish our findings to be clinically relevant to human diseases of the heart, and the use of other less sentient species, such as reptiles, fish and amphibians, is usually not appropriate for the main study animal as their hearts differ significantly from mammalian hearts. Nevertheless, we have utilised the zebrafish which are naturally tolerant to hypoxia to understand adaptive responses.

Reduction

Explain how you will assure the use of minimum numbers of animals.

Experimental design has been discussed with, and approved by, our statistical advisor. In order to minimise the number of animals required, sample size has been estimated for each investigation based on existing published data and the use of power analysis. These estimates will be updated and recalculated throughout the project as we generate new data.

Refinement

Explain the choice of species and why the animal model(s) you will use are the most refined, having regard to the objectives. Explain the general measures you will take to minimise welfare costs (harms) to the animals.

Experiments concerning rats:
We have chosen the rat as our main experimental species for several important reasons:

1. Rats reach reproductive maturity quickly and they have a short lifespan (2 years) which allows the long-term effects of prenatal hypoxia to be studied within a reasonable timeframe

2. The rat model of prenatal hypoxia is well-established; there is a wealth of information to base our investigations on.

3. My collaborator has already determined that antioxidant therapy reverses the negative effects of prenatal hypoxia which gives us an opportunity to develop drug treatments to protect the heart.

Steps to minimise welfare costs to animals:

1. It is not possible to house pregnant rats in groups because we need to monitor food and water intake during the study; but once pups have been weaned, rats will be housed in stable, compatible groups.

2. The following parameters will be measured during the protocol to ensure animals remain within the outlined severity limits: Body weight, body condition scoring (BCS), food and water intake and cardiovascular status.

3. Control animals not subjected to any procedures will be used as a benchmark for normal changes in these parameters.

4. For moderate levels of prenatal hypoxia (below 13% oxygen), rats will first be put into the chamber at normal oxygen levels (21%) for 24 hours, and then oxygen will be reduced slowly (over another 24-hour period) to avoid shock.

5. The following rules will be applied to rats transported to collaborator establishments;

- Prior to transport, all rats will undergo a health check by local NVS and at least 2 of the rats will be screened for viruses and pathogens.

- Rats will be transported according to the NC3R's best practice for animal transport, and in line with UK legislation according to the The Welfare of Animals (Transport) (England) Order 2006. We will use a reputable courier who is regularly used by the establishment.

- Upon arrival at the collaborator's establishment, another health check will be made by the collaborators NVS

6. When rats are subjected to dietary interventions, blood pressure will be measured regularly to make sure they are not at high potential risk of an adverse cardiovascular event

<u>Experiments concerning fish:</u>

Similar to rats, fish have a short lifespan which make the suitable for longitudinal studies, but they also routinely experience developmental hypoxia in the wild, and they are known to mount adaptive responses to hypoxia. Studying these species will allow us to identify adaptive mechanisms which could be manipulated in mammalian hearts to provide protection against hypoxia.

Steps to minimise welfare costs to animals:

Zebrafish are extremely hypoxia tolerant, so we do not expect any adverse effects from hypoxia exposure. Nevertheless, we will regularly monitor the animals for signs of illness or disease.